Rapid screening of melamine in dried milk without destroying the samples by Raman spectroscopy

SHORT COMMUNICATION

Rapid screening of melamine in dried milk without destroying the samples by Raman spectroscopy

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Abstract Melamine is a raw ingredient for plastics, but it is frequently misused by adding it to food to give the false impression of a high protein content, since the molecule has a very high nitrogen content. Samples of dried milk obtained from 5 manufacturers containing varying levels of melamine were illuminated by a small cross-section of a laser beam and the scattered light was examined. The presence of melamine in contaminated milk could be immediately detected without any chemical or physical alteration of the milk by interpreting its Raman scattering spectra. Among its many Raman bands, the intense 676 cm^{-1} band was the most useful for detecting melamine; detection limit was about 1% (w/w). Because this method does not extract melamine from the dried milk sample, it reduces the risk of error that may occur during the extraction and interaction with chemical reagents. The method provides a very rapid screening test for melamine-adulterated dried milk in food chemistry and forensic toxicology.

Keywords Melamine・ Raman spectroscopy・ Dried milk・ Adulterant・ Screening test

Introduction

Melamine, 1,3,5-triazine-2,4,6-triamine, is a trimer of cyanamide, $NC\text{-}NH_2$. With formaldehyde, melamine produces a resin that is a very good plastic and it is used extensively in commercial products.

In 2007, about 1500 domestic pets (dogs and cats) in the United States died as a result of consuming Chinese-made canned foods that were tainted with melamine. In 2008, about 54000 infants were poisoned by drinking contaminated milk formulae in China and five of them died. Later, it was found that melamine had been deliberately put in many dairy products produced in China for export to other countries [1]. Normally, protein content in food is expressed by nitrogen content. Looking at the chemical formula for melamine ($C_3H_6N_6$), the high nitrogen content is obvious. In the tainted pet food, melamine was detected in glutene, a wheat protein. The manufacturer of the pet food fooled customers into thinking the canned food was high in protein content; similarly, the tainted milk also gave the impression that it contained high protein content. Milk contains lactose and various proteins. Since lactose does not contain nitrogen, adding melamine to the tainted milk fooled the customers into perceiving a high protein content. The motivation for this practice was to illegally obtain profit, disregarding business morals [2].

Analysis of melamine has been carried out by gas chromatography (GC) [3, 4], GC-mass spectrometry (MS) [5,6], liquid chromatography (LC) [7-9], LC-MS (-MS) [10-14] and Raman spectroscopy [15,16]. Among these methods, the final identification can be achieved by MS and Raman spectroscopy. For MS measurements, careful pretreatments of crude samples for purification of the target compound(s) are required. For Raman spectroscopy, such pretreatments are

sometimes not necessary, subject to the absence of interfering peaks or fluorescence in a wave number range where the target compound peak is expected to appear [17]. In the preexisting reports using Raman spectroscopy, they dealt with pure melamine[15] and melamine in gluten, chicken feed, cake, and noodles[16]. In this article, we present that melamine in dried milk can be detected immediately without destroying it by merely focusing laser light and analyzing the reflected Raman scatter.

Materials and Methods

Materials

The standard melamine, melamine sulfate and indene were purchased from Wako (Osaka, Japan). Five brands of dried milk used in this study were: “Hohoemi” from Meiji Dairies (Tokyo, Japan); “Dry Milk Hagukumi” from Morinaga Milk Industry (Tokyo, Japan); “Pure” from Snow Brand Milk Products (Sapporo, Hokkaido, Japan); “Lebensmilk Haihai” from Wakodo (Tokyo, Japan); “Neomilk Sukoyaka” from Bean Stalk Snow (Sapporo, Hokkaido, Japan). They were purchased at a local grocery store.

Preparation

Each standard melamine, melamine sulfate, or each brand of dried milk was crushed into fine powder in a mortar. The fine-powdered melamine and each dried milk were mixed to make 10, 3, 1, 0.3 or 0.1% (w/w) sample. About 60 mg of each sample mixture was placed in dies (8 mm diameter) and hand-pressed to make thin pellets.

The pellets were fixed on slide glass with Blu-Tack to be subjected to the Raman spectroscopy.

Raman measurements

Raman spectra were measured on a HoloLab5000-785 Raman spectroscopy system (Kaiser Optical Systems, Ann Arbor, MI, USA) equipped with a measurement probe (HoloProbe-785, Kaiser Optical Systems), to which a hand-made light microscope for locating the beam spot was attached. The excitation wavelength of the laser was 785 nm; the spectral measurements were conducted with a 50 s (5 s x 10 times) exposure time and 80 mW laser power. The spectra obtained were corrected by measuring the spectrum of indene.

Results

Standard melamine

Two standards were examined first: melamine and melamine sulfate. The two samples essentially showed identical spectra (Fig. 1). Of the many spectral bands, the peak at 676 cm^{-1} (Fig. 1A) is the most prominent; this peak was used to monitor the melamine in the milk samples.

The assignments of various bands were based on information in several books [17-19]. There are three low-frequency bands (120-156 cm^{-1}) (Fig. 1A). The exact assignment of these bands is difficult because of the lack of reference spectra of similar compounds, but it is very likely due to a breath vibration of the ring containing C and N. The bands from 378 cm^{-1} to 985 cm^{-1} are likely to originate from various

CNC bending vibrations and C-N-C symmetrical stretching vibrations (985 cm^{-1}). The 1444 cm^{-1} to 1659 cm^{-1} small bands are C=N stretching vibrations and NH bending vibrations. The broad band at 3126 cm^{-1} is most likely due to NH stretching vibrations.

Detection of melamine from dried milk

Before adding melamine to the milk samples, the spectra of dried milk were examined. Figure 2 shows a typical example of spectra obtained from blank dried milk samples of five brands currently sold in Japan. All samples showed similar spectra with no notable peak around 676 cm^{-1}. The prominent peak at 2900 cm^{-1} is obviously due to various CH vibrations of milk proteins that did not appear in the melamine spectra.

As the concentration of melamine increases, the bands characteristic to melamine became clear. Notable were the low-frequency bands less than 159 cm^{-1}, the 676 cm^{-1} main peak, and the 3126 cm^{-1} band; all became more apparent as the concentration of melamine is increased. For detection purposes, the 676 cm^{-1} band was the most useful (Fig. 3).

The peaks at 676 cm^{-1} were measured for 10, 3, 1, 0.3 and 0.1% melamine in dried milk as shown in Fig. 3. The detection limit was about 1%.

Discussion

The LD_{50} of melamine is 3000 mg/kg in mice indicating its extremely low toxicity; it is almost a non-toxic substance. Therefore, the question that arises is how a non-toxic substance causes death in humans and animals. First, it is notable that the LD_{50} is only applicable to acute toxicity [19]. Melamine toxicity is not of the acute type; rather, it is a short-range chronic toxicity. Melamine is metabolized to cyanuric acid in the body after ingestion. Cyanuric acid has three keto groups and melamine has two amino groups. Two of the keto groups form hydrogen bonds with the two amino groups, forming a highly insoluble material that sediments all over the kidneys (Fig. 4). The formation of the melamine-cyanuric acid complex may be compared to a kidney stone; however, it is important to realize that it is different from a natural kidney stone. Natural kidney stones can be of many types, including calcium phosphate, uric acid, cysteine, and oxalic acid types. It is rare, but occasionally protein-type kidney stones are detected.

Our current results suggest that Raman spectroscopy is a very useful technique for detecting the presence of melamine in milk without any chemical treatment of the sample. Moreover, the analysis does not involve any extraction, purification, or chemical reagents. It is a rapid and non-destructive technique for detecting melamine in milk.

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Legends for Figures

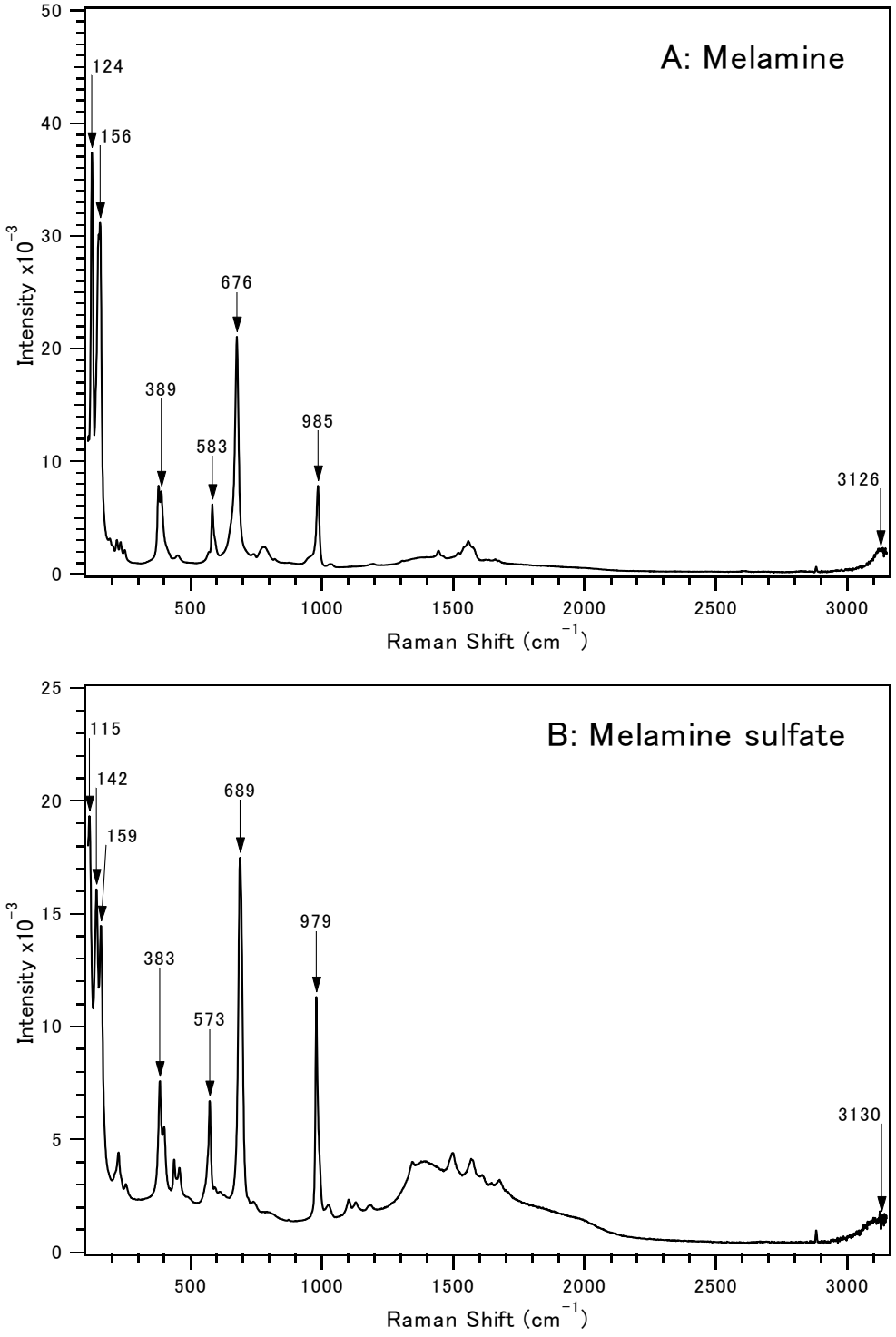

Fig. 1A, B Raman spectra of the standard melamine (A) and melamine sulfate (B)

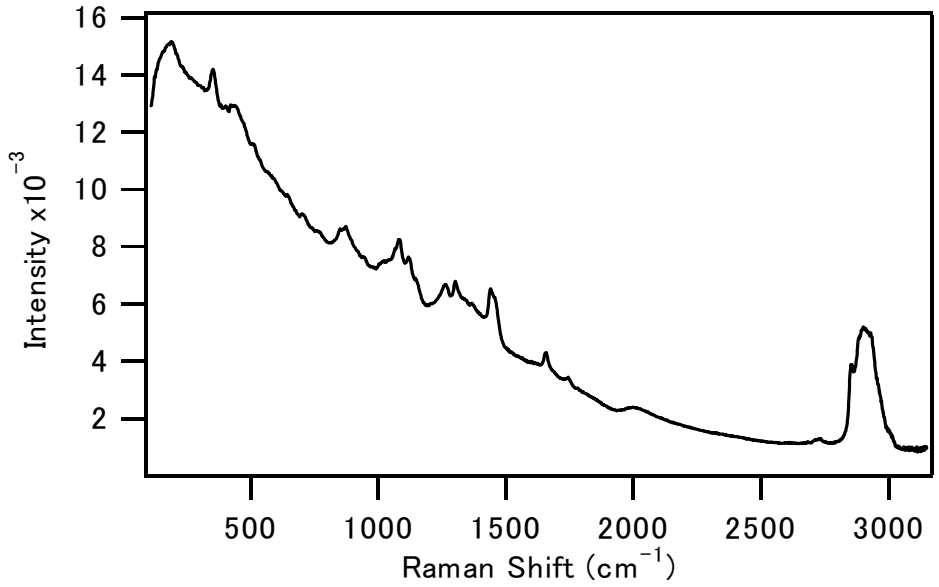

Fig. 2 A typical Raman spectrum of blank dried milk obtained in Japan. Five brands of dried milk were tested and very similar spectra were obtained for all of them. This sample was obtained from Morinaga Dry Milk Hagukumi

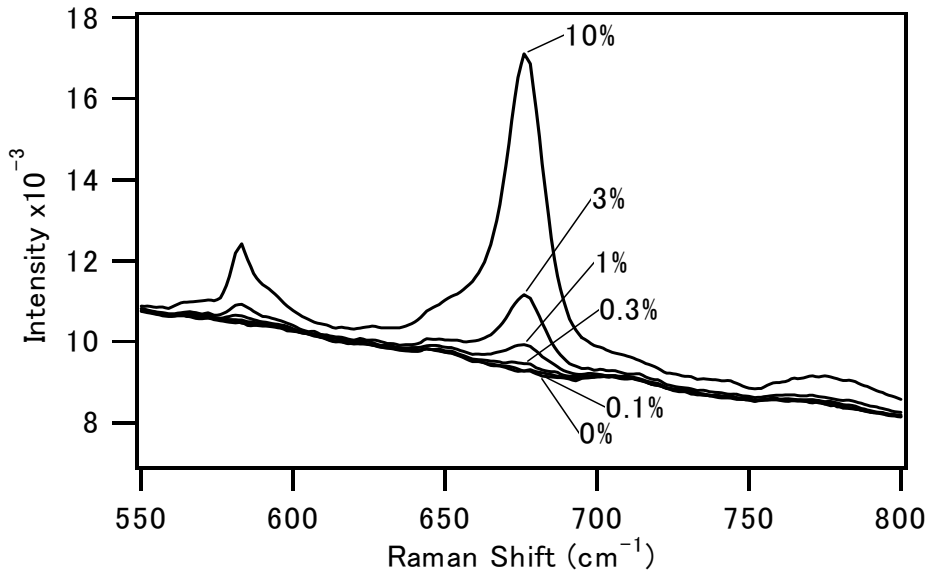

Fig. 3 Raman spectra of dried milk samples spiked with different concentrations of melamine. The peak maxima appeared at 676 cm^{-1}.

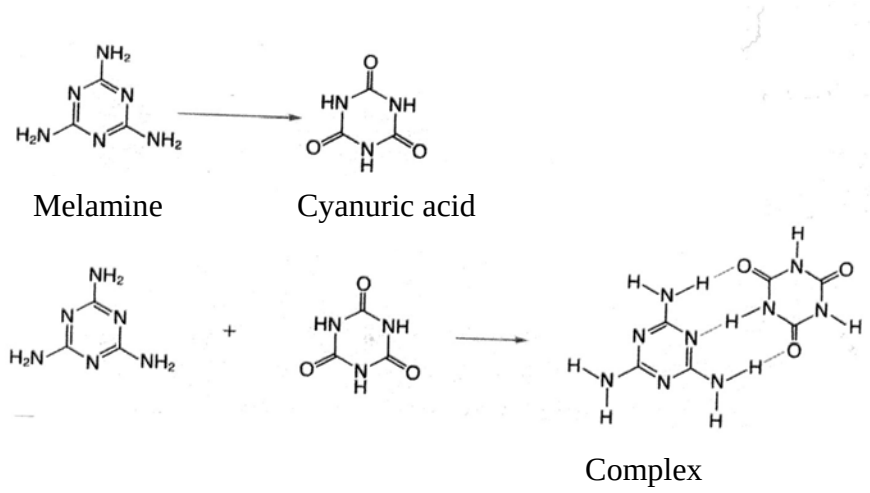

Fig. 4 Metabolism of melamine and its complex formation of melamine-cyanuric acid that precipitates in the kidney.